IN SITU LOCALIZATION OF ORGANIC MATTER IN SOILS

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ABSTRACT

Three main size classes of organic matter in soils are ingested by soil animals: (1), multicellular plant and animal remnants (5000 - 50 μm in diameter); (2), microorganisms (50 - 0.3 μm); and (3), subcellular fragments (1 μm - 10 nm). Ultracytological techniques locate particular reactive sites (polyphenolic, acidic and neutral carbohydrates, enzymes) on soil organics in situ *in natural soil fabrics. This allows investigation of morphological and biochemical characteristics of organic resources available to soil animals and changes involved in organic matter transformations as materials pass from class 1 to class 3. This involves both breakdown of cellular materials and biosynthesis of new organics by microorganisms. In particular, I address such questions as: where in soils are the different size and biochemical types of organic matter located with respect to soil minerals and soil microorganisms; how are plant and animal tissues reduced to deposits of submicron and even macromolecular sizes; how do biodegradable materials (e.g., polysaccharides) escape microbial degradation; how do the physically and chemically protected organic matter deposits differ in structure, biochemistry and location; how do microbial products bind soil components into stable aggregates and how do these subsequently break down; how do these changes affect their availability and nutrient status for soil animals?*

RÉSUMÉ

Il existe dans les sols trois catégories principales de grosseurs de particules organiques qui sont ingérées par les animaux endogés: 1) des restes d'animaux et de plantes multicellulaires d'un diamètre variant de > 5000 à 50 μm, *2) des microorganismes d'une taille variant de > 50 à 0.3* μm, et 3) des fragments subcellulaires variant de 1 μm *à 10* μm. *Des techniques d'ultracytologie ont été utilisées pour localiser des sites réactifs particuliers (hydrates de carbone polyhénoliques, acides et netures, enzymes) sur des particules organiques en place dans les structures naturelles de sols. Cette approche permet d'étudier les caractéristiques morphologiques et biochimiques des ressources organiques disponibles pour les animaux endogés, de même que les changements qui se produisent durant la transformation des matières organiques alors que les matériaux passent de la catégorie 1 à la catégorie 3. Cette transformation comprend la décomposition des matériaux cellulaires et la biosynthèse de nouvelles substances organiques par les microorganismes. Plus particulièrement, je pose les questions suivantes: où dans les sols se situent les différentes grosseurs et les différents types biochimiques de matière organique par rapport aux minéraux et aux microorganismes? Comment les tissus animaux et végétaux sont-ils décomposés en des dépôts de taille submicroscopique ou même macromoléculaire? Comment des substances biodégradables (comme les polysaccharides) échappent-elles à la dégradation microbienne? Comment des dépôts de matière organique protégés physiquement et chimiquement diffèrent-ils dans leur morphologie, leur biochimie et leur position? Comment les produits résultant de l'action microbienne lient-ils les composantes du sol en des agrégats stables, et comment ceux-ci sont-ils par la suite décomposés? Comment ces changements affectent-ils leur disponibilité et leur qualité nutritive pour la faune du sol?*

INTRODUCTION

Little is known about organic matter in its native state in natural soil fabrics. Nearly all information on soil organics comes from studies of materials which have been chemically or physically extracted from the soil and introduced into a quite different biophysical and biochemical milieu for characterisation and quantification. Detailed knowledge of the structure, biochemistry, microbiology and location of organic materials *in situ* in soil fabrics is of great importance to determine where the various phases of organic matter mineralization occur and where nutrients are available to soil animals.

Ultrastructural studies of soil organics are concerned with particles from c.100 μm to 10 nm, so in investigations of the processes of organic matter decay and nutrient recycling, ultrastructural studies bridge the gap between materials studied by the soil micromorphologist and those studied by the soil biochemist.

Organic materials in soils are infinitely variable in their structure and biochemistry, depending on their source and the amount of microbial decay and chemical weathering they have undergone. They range in size and ultrastructural complexity from histons of plant and animal tissues which are structurally almost unchanged from their living condition down to fragments of almost macromolecular size which have undergone profound morphological and biochemical transformations (Foster and Martin, 1981).

Although modern SEMs will take specimens weighing up to 1 kg, at useful magnifications (say 10,000x) the amount of material represented in individual electron micrographs is ($<10^{-6}$cc [see below]) so that ultrastructural studies are limited to the finer details of effects of soil animals on soil structure. Similarly the animals observed in electron micrographs of even moderate magnifications must be restricted to microorganisms - ciliates, flagellates, amoebae *etc.* Although these are very numerous in soils (10^6 /cc Darbyshire and Greaves, 1967), their effects on soil organic processes were until recently, much neglected. In this paper I examine the physical and biochemical environments near, and the location and structure of organic materials available to soil animals, over a wide range of sizes.

METHODS

Electron optical methods

The study of soils by electron optical methods includes the use of conventional- (CTEM) and scanning-transmission electron microscopy (STEM), scanning electron microscopy (SEM) using either secondary electron, or back scattered electron detection (BSEI), and electron probe microanalysis (EPMA) using energy (EDXRA) or wavelength (WDXPA) dispersive X-ray analysis, (Bisdom, 1983). Unfortunately, biological materials in soils consist of exotic molecular species made up of a rather limited number of kinds of atom so elemental analysis by EPMA is not widely applicable to biological problems (Hayes, 1980). Conventional EPMA instruments do not easily detect light elements such as C, N, and H, of which most organics of biological importance are composed. EPMA has been used however to investigate the distribution of P, K, Ca and Mg in roots and rhizospheres (Tan and Nopamornbodi, 1981). This review is confined to TEM and SEM studies of soil organics *in situ* in natural soil fabrics: Smart and Tovey (1981) and Bisdom (1983) provide excellent reviews of the submicroscopy of the mineral components.

Physical dimensions of specimens

Theoretically, the early stages of organic matter broken down to particles of micron size can be studied by light microscopy using thin sections of soil but practically, section thickness and the presence of opaque minerals and organic matter limits resolution to about 5-10 microns. The use of conventional heavy metal staining methods and TEM of ultrathin sections has allowed the detection of particles down to nanometer sizes *in situ* in natural soil fabrics (Foster and Martin 1981). At these sizes, however, except where the organics have a distinctive structure (membranes, microfibrils *etc.*) it is difficult to distinguish between organic and inorganic particles. Bisdom (1983) summarises the application of more sophisticated techniques for the identification of materials in soil samples, (ion microprobe mass analysis (IMMA), secondary ion mass spectrometry (SIMS), laser microprobe mass analysis (LAMNA *etc.*) which may be useful in distinguishing between organic and inorganic amorphous materials. Such sophisticated techniques are not generally available to soil scientists, however, so I have used ultracytochemical techniques to investigate the biochemical properties of the small fragments of organic matter *in situ* in soil fabrics.

SEM specimens may be up to 1 kg in size, but the area sampled in an electron micrograph depends on magnification and is usually quite small. For CTEM and STEM specimen size is limited by the distance fixatives and embedding media will penetrate. Blocks of soil only 0.5 - 1 mm cubed give the best results. The actual sections are 0.5 x 0.5 mm and 0.1 μm thick.

Physical and chemical stabilization of the soil fabric

Various components in plant and animal materials are naturally held together to form tissues. Similarly, interlocking crystals hold sections of rock samples together. Soils, on the other hand, are composed of randomly disposed and relatively widely spaced minerals, organic fragments and soil microorganisms lying free or only loosely interconnected. Hence, except for apical and sub-apical rhizospheres where the soil fabric is embedded in mucigel (Foster, 1981b; Campbell and Porter, 1983), before ultracytochemical analysis can begin, the soil must be stabilized both physically and chemically. Physical stabilization prevents relative movement of soil components during biochemical processes. It is achieved by embedding the soil sample in an amorphous gel such as gelatine or agar. Chemical stabilization prevents the loss of soluble components (lipids, low molecular weight gels *etc.*) during solvent exchange dehydration and is achieved with cross-linking agents such as aldehydes and/or polyvalent metals such as lanthanum. For ultramicrotomy the soil must be dehydrated and embedded in plastic (see Foster and Martin, 1981; Smart and Tovey, 1981 for details of techniques for soil specimens).

Ultracytochemistry

Ultracytochemistry is the detection and/or identification of (usually organic) materials in biological tissues by electron optical techniques. Here I use the term for any organic deposit whether part of a cell or free in the soil fabric. Ultracytochemistry has been used in biology for more than 30 years. At its simplest, it merely consists of adding solutions of heavy metals (typically Os, Pb, U) to soil samples. These react with, or are absorbed onto organics so that in ultrathin sections (50 nm - 100 nm thick) where they were previously electron transparent (and therefore invisible) they become electron opaque and so readily detectable.

Techniques for specific complex molecules.— Ultracytological techniques have the advantage that specific complex macromolecules with well defined biochemical properties can be detected and located *in situ* in a soil fabric section with a resolution measured in

nanmometers. For example, histochemical methods have been devised for examples from every major group of enzymes (Hayat, 1975). All the methods used here are from Sexton and Hall (1978).

Techniques for particular chemical groups.— Hayes (1980) has suggested that examples of pure biochemicals such as particular lipids, proteins, polyphenols and carbohydrates which can be isolated from living cells may be very uncommon in soils. Instead, uncontrolled enzymatic reactions during cellular autolysis and chemical reactions between the lysates, soil minerals and pre-existing soil organics, produce random combinations of these materials to form unique complexes (*e.g.,* humates) which no longer closely resemble the biochemicals found in living tissues.

Fortunately most ultracytological reagents detect particular reactive groups *e.g.,* 0s04 reacts with phenolic hydroxyls, alkyl groups, sulphydryl groups (for groups derived from plant residues see Bland *et al.*, 1971) so that conventional aldehyde/0s04 treatment detects plant cell wall remnants and humic material. The acidic polysaccharides of mucins, bacterial and fungal slimes and root mucilages are stained with ruthenium red or lanthanum hydroxide (Foster, 1981b).

Where they are not present naturally, specific reactive groups can sometimes be experimentally generated by chemical pretreatments. Thus neutral carbohydrates are detected by partial lysis with periodic acid to generate aldehyde groups which are then labeled directly with silver methenamin (PAMS) (Pickett-Heaps, 1967) or *via* thiosemicarbazide with silver proteinate (PATSP-Thiery, 1967). These methods give electron-dense deposits with a characteristic structure so that carbohydrates can be detected even against a background of soil mineral fragments, and have been used to demonstrate carbohydrate coatings in clay fabrics (Foster, 1981a). Similarly, complex epoxides which occur in leaf (Brown and Holloway, 1981) and root cuticles are detected by iodenation and addition of suitable silver compounds. Hence a wide range of biologically significant materials can be positively and specifically located in or on organic particles in soils.

Detection by specific removal.— Other organic materials can be located by noting sites where staining disappears if the section is treated with a solvent or an enzyme. Thus Heritage and Foster (in press) identified native S grains in sulphur bacteria in sections of waterlogged soil by their solubility in CS2. Since specific, highly purified enzymes are available commercially this has some potential, but does not seem to have been applied to soil components other than recognisable tissues (*e.g.,* mycelial strands, Foster, 1981c).

SOURCES OF ORGANIC MATTER IN SOILS

Newly deposited materials

The most common material entering soils is carbohydrates derived from leaves, branches, bark fragments and fragments and floral parts (especially pollen), and from root mucilages, exudates, and ephemeral root tissues.

Materials from aerial organs.— Depending on the depth from which the sample is taken, leaf fragments retain much of their characteristic cellular structure even though their tissues have been invaded by microorganisms (Plate 1a, b). Autolysis before leaf fall results in the loss of cytoplasm in many cells, and release of vaculolar polyphenolics causes the cell walls to be impregnated with materials which make them more electron dense after heavy metal staining than would occur in the live leaf (Plate 1c). Pine needles often contain extensive deposits of polyphenols which partially occlude cell lumens and stain the cell walls (Plate 1a, b). Most of

the carbohydrates are quickly removed by microorganisms so the cell walls collapse onto the vacuolar contents (Plate 1d). Eventually only much convoluted, lignified cell wall layers remain and the origin of the material becomes indeterminate (Plate 1e).

Materials from Roots.— One of the more interesting facts to emerge in the last 10 years is that roots deposit large amounts of organic matter into the soil whilst they are still functional. Up to 30% of the photosynthate reaching the root may be released into the rhizosphere (Barber and Martin, 1976; Martin, 1977; Martin and Puckridge, 1981) as gels, exudates and lysates (see Rovira *et al.*, 1979, for definitions). Most classes of plant metabolites (sugars, amino acids, vitamins, proteins, lipids, hormones *etc.*) have been isolated from root exudates (Rovira, 1965) but these are not preserved in preparation for electron microscopy. However they support bacteria and fungi (Plate 2a, b) which colonise the complex carbohydrates secreted by the root in the form of mucilages and proteins (*e.g.*, enzymes). In some cereal crops more carbohydrate may enter the soil as root mucilage than is stored in the grain as starch, (Samtsevitch, 1965). For example White (1983) estimates sloughed cells and gel amount to 3.5 tonnes/ha/yr for wheat.

Direct evidence from electron microscopy (Plate 2a) (Foster, 1981b; Foster *et al.*, 1983; Campbell and Porter, 1982) and theoretical calculations (Newman and Watson, 1977; Gardner *et al.*, 1983) suggest that these materials are mainly confined to the immediate vicinity of the root (0 - 150 μm). Tan and Nopamorabodi (1981) found a sharp break in P distribution between 200 - 300 μm from the root surface which may also indicate the outer limits of the rhizosphere gel. Using quite independant ultrastructural techniques Campbell and Porter (1982) and Foster (1981b) showed that there was an inner layer of mucilage near the cell surface (Plate 2a, b) which was much more dense than that 20 - 50 μm away; so, there may be partition of root products with distance from the root on the basis of molecular weight. Carbohydrates are neither preserved nor stained by conventional biological preparation techniques (Foster and Martin, 1981) but the acidic carbohydrates are preserved and stained by lanthanum hydroxide (Plate 2a) and the neutral carbohydrates by the PATSP (Plate 2b) and PAMS reactions (Plate 2c). These electron micrographs of known plant materials serve to calibrate these cytological tests for non-rhizosphere soils to be presented later.

All these root-derived organics are available to those animals such as collembolans, nematodes and enchytraeid worms which browse along roots (Head, 1967). At first, root mucilage may be enclosed by a cuticle (Greaves and Darbyshire, 1972), but this is soon ruptured (Foster, 1981b) allowing the gel to penetrate into the soil fabric. Mucilage appears to be a true gel, allowing water and ions to diffuse through it in a manner not significantly different from that in free water (Greenland, 1979). Although most of the root gel is secreted by the root cap, epidermal cells and root hairs also secrete mucilage. In drying soil, this may hold soil firmly to form a rhizosheath (Wallstein and Pratt, 1981). At first, colonies of bacteria develop in the soil surrounding the root in response to exudates penetrating the soil fabric. Later, bacteria and fungi attack the gel, especially along the grooves between the epidermal cells, leaving lysis holes (Plate 2a, b, c) in the mucilage.

In some semi-permanent grasslands, 53 - 98% of the standing crop is below the ground, and some grasses show a 100% root turnover each year (Dickinson, 1982) amounting to 5000 kg dry matter/ha/y (Whitehead *et al.*, 1980), so root tissues may be a considerable component of the annual organic matter input into soils. Little wonder then that Curry and Ganley (1977) found 89,000 microarthropods/sq meter, 80% being acarines and collembolans. Recent work has shown that death of the root cortex is a normal phenomenon unassociated with disease (Henry

and Deacon, 1981, van Vuurde *et al.*, 1979). Materials released by the death of cells causes a population explosion amongst the root surface microorganisms. Thus van Vuurde *et al.* (1979) found that in root segments 4 - 5 days old, where 35 - 45% of the cells were dead, supported a 1 - 4% microbial cover. When 45 - 75% of the cells were dead, (segments 7 - 8 days old), microbial cover increased to 8% of the root surface area.

Animals and their remains.— Most larger animals (here > 1mm!) escape during preparation, though nematodes may be observed in dead root fragments (Plate 3a), and soil ingesting animals recognised by clay and organic particles in their gut (Plate 3d), are occasionally encountered. The most common animal remains in ultrathin sections of soil are exuviae fragments and cast off appendages (Plate 3b, c, e, f). These are identified by their characteristic structure, ultrastructure and histochemical reactions (Foster, 1978; Foster and Martin, 1981). Live animals most commonly observed in soil sections are microorganisms such as amoebae and cilliates, (Plate 3 g, h) *etc.*

The abundance of soil animals in the surface layers of soils means that fecal pellets are of common occurrence, but they are not necessarily all derived from soil inhabiting animals; in some forests there is an almost continuous rain of pellets released by phytophagous insects in the canopy. Fecal pellets are recognised in SEM by their characteristic size and shape and some can be attributed to particular species. In TEM, fecal pellets and their fragments are easily recognised because their high enzyme and/or mucin contents make them stain strongly with heavy metals. The chaotic arrangment of their contents is characteristic, and often they contain cellular materials which are so little altered by digestive processes that cellular organelles (such as the thylakoids of chloroplasts) can still be recognised (Plate 3i). Many pellets contain bacteria, or support bacterial colonies in the surrounding soil (Foster *et al.*, 1983).

Further microbial breakdown.— Once incorporated in the soil, rapid degradation of tissues occurs through the action of plasmodia, fungi and bacteria. In turn, fungal hyphae are broken down by bacteria (Kilbertus and Reisinger (1975). Resins and polyphenolics from pines may be deposited in the soil (Foster and Marks, 1967). Kilbertus and Reisinger (1975) examined the stages in breakdown of leaf litter at the ultrastructural level. In clay soils most bacteria are associated with organic matter (Plate 4a - d). The larger bacteria are associated with cell wall remnants which still contain carbohydrate (electron transparent materials) (Plate 4a, c), but throughout the humified organic matter and even in the mineral rich parts of the fabric, there are many small microorganisms, many 3 μm in diameter (Plate 4a, b, d). Gradually organic and mineral soil components become intimately mixed. Firstly clay platelets become absorbed onto the gels secreted both by roots (Plate 2c) and bacteria (Plate 5a, b). Secondly fungal hyphae, root hairs and pieces of plant cell walls tens of microns in length become enclosed in extensive clay fabrics several microns thick (Plate 5c, f, g). The same is true for amorphous materials (Plate 5d, e, h, i). These materials will be physically protected from microbial attack until they are ingested and broken open in the alimentary tract of soil animals. They constitute part of the physically protected organic pool in soils.

Secondary sources of organic matter in soils

Microbial tissues and their secondary metabolites.— All the materials previously mentioned are further modified by microorganisms. Bacteria, actinomycetes and fungi are the most commonly encountered microflora and their lytic activities may be a prerequirement before tissues become available to soil animals.

Plate 1. Primary sources of soil organic matter – leaves. – (a). Although most of the tissue has been replaced by microorganisms, the thickness of the cell wall indicates that the tissue was a leaf epidermis. (b). Detail of (a). The former cuticle is occupied by hyphae. The cell lumen is partly occluded by tannins(T). (c). Later stage of decay-only polyphenol rich cell wall remnants remain. (d). Most of the electron transparent carbohydrates have been removed from the cell walls so the cells have collapsed onto the vacuolar tannins(T). (e). Highly decomposed leaf tissue from a waterlogged pasture. Only distorted, humified cell wall layers remain.

Plate 2. Primary sources of organic matter– roots.– (a). Lanthanum hydroxide reacts with substituted carbohydrates in the root surface mucilage (RM) which has been partially lysed (L) by soil bacteria. The mucilage holds cell wall remnants(W), clay particles (C) and a colony of bacteria (B) onto the root surface. (b). Neutral carbohydrates in the root mucilage (RM) are demonstrated by the PATSP technique. The gel and exudates support colonies of bacteria (B) near the root. Quartz grains (Q) shatter during ultramicrotomy. (c). The cell wall of both root (W) and bacteria (B) are intensely stained by the PAMS reaction but the root gel is only lightly stained. Clay particles(C) and quartz grains become embedded in the gel which is locally lysed by bacteria (L). (d). Even where root mucilage (RM) is separated from the root (W) by clay it can be recognised by its characteristic granularity and reaction with ruthenium red/0s04 complex. (e). Extensive decay by colonies (B) and individual microorganisms (arrows) leads to the collapse of the root tissue.

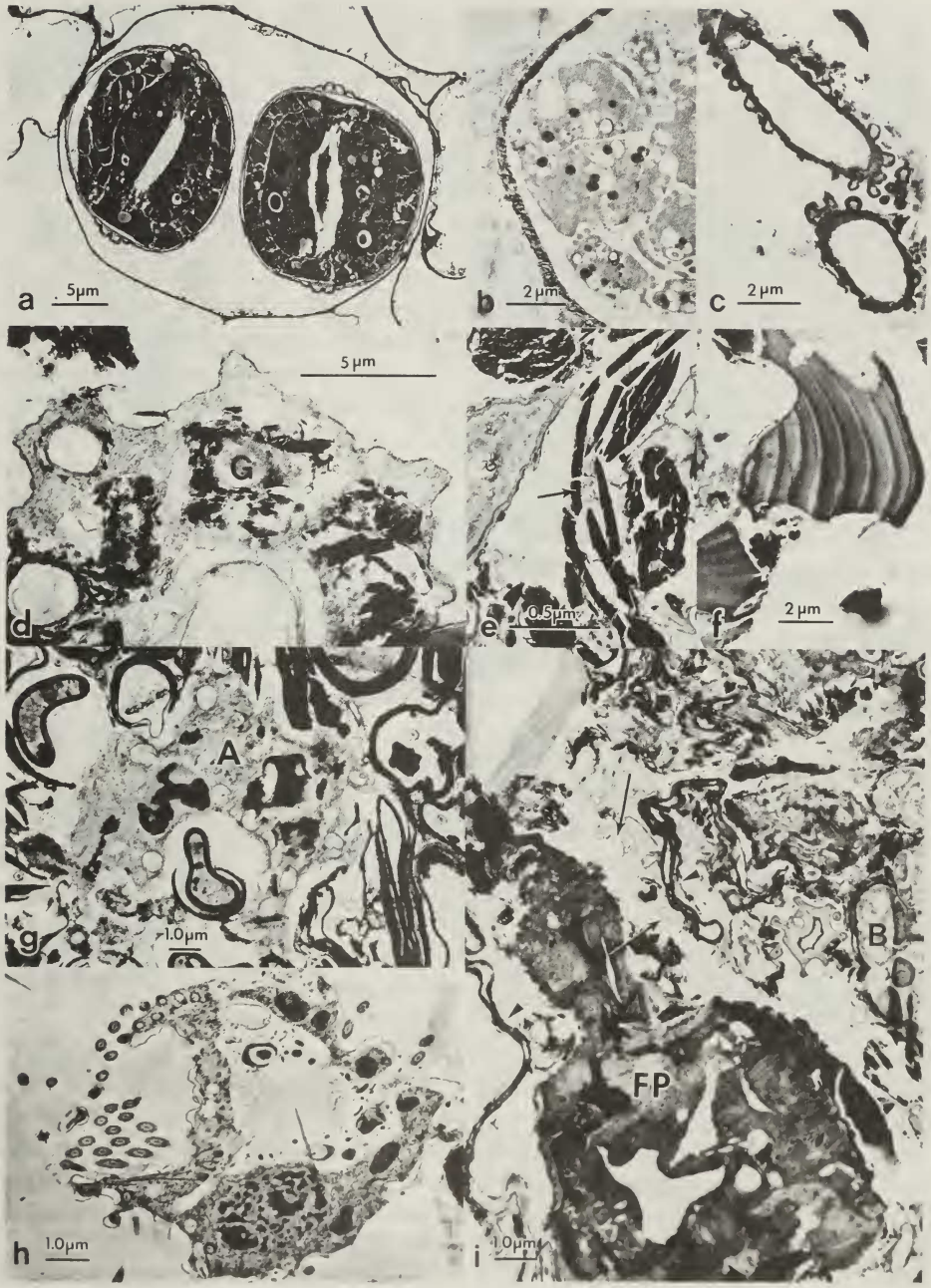

Plate 3. Soil animals and their remains.– Soil animals which play a major role in organic matter mineralization. (a). Nematode (?) in dead root fragment. (b). Appendage with live cells. (c). Empty appendages. (d). Soil ingesting nematode (?) showing several sections of the gut (G) with clay particles and organic matter. (e), and (f). Cast off and partially broken down insect parts. (g). An amoeba (A) in an organic rich surface soil. (h). A ciliate attached to a rhizomorph. (i). A fecal pellet (FP) recognised by its chaotic and electron dense contents, supports bacteria (B). Note nearby cell wall remnants (arrow heads) and membrane systems (arrows).

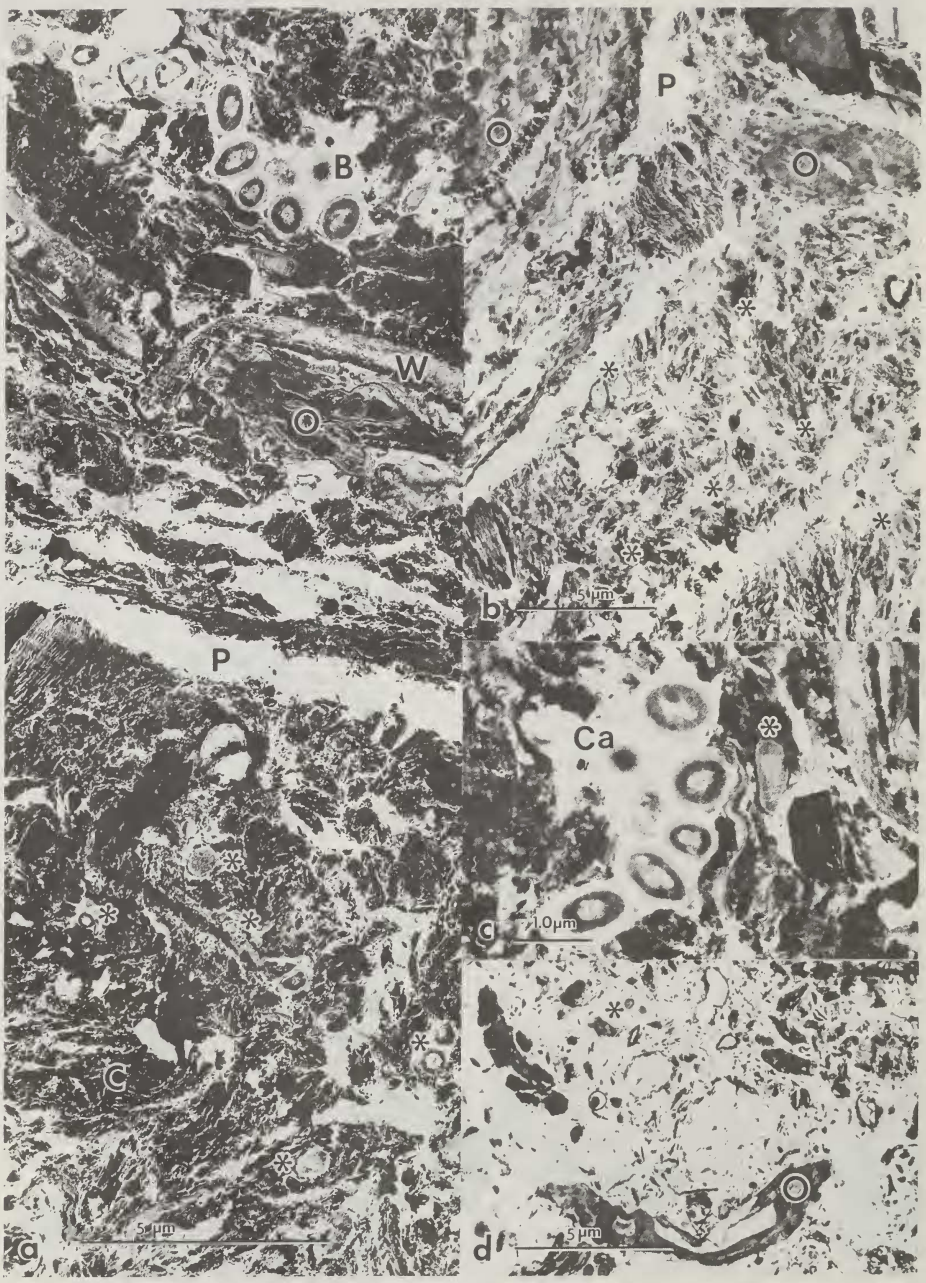

Plate 4. Soil fabrics with microorganisms and organic matter remnants.– (a). Bacteria (B) are usually associated with organic matter which still contains carbohydrate (eg. cell walls W). Other highly lignified and convoluted organic matter (0) does not support bacteria, but there are numerous microorganisms (0.3 μm diameter) scattered throughout the clay (*). P is a pore about 1 μm in diameter. (b). Similar to (a). (0) is amorphous organic matter. Again there are numerous small soil bacteria in the clay (*). (c). Detail of (a) showing bacteria enclosed in capsule material (Ca) which is not stained by 0s04.(*) indicates a capsule-less bacterium enclosed in humified organic matter. (d). A less consolidated clay fabric containing remnants of cell wall remnants (0) and an occasional microorganism (*).

Skinner *et al.* (1952) estimated that conventional plating techniques used by soil microbiologists only detected between 0.1 and 1% of the organisms present. Morever according to Bae *et al.* (1972) more than 63% of soil microorganisms are less than 0.3 μm in diameter and so not readily seen by light microscopy. Campbell and Rovira (1973) showed that because bacteria were enclosed in gel they are not readily detected by SEM and TEM of thin sections is necessary to clearly see all the microorganisms in a soil and to determine their probable viability (as indicated by their cytoplasmic ultrastructure).

The abundance of mucilages and exudates in young rhizospheres stimulates the growth and division of bacteria, fungi and actinomycetes both in and around the root. The more readily available exudates are used by less specialised bacteria such as the fluorescent pseudomonads which are particularly common in rhizospheres. A host of fungi, epiphytes, symbionts, cortical and stelar parasites, then colonise the roots and rhizospheres. These are followed by saprophytes, and together with bacteria, they remove the less resistant polymeric materials. Using electron microscopy, Foster and Rovira (1976) showed that a consortium (White, 1983) of different microorganisms were involved in removing different chemical fractions of the cell wall. Later, actinomyctes and bacilli become more abundant; these are able to attack more resistant materials such as lignified secondary walls of tracheids, sclerenchyma *etc.* Rhizosphere microorganisms reach population of 1.2^{10} E+10/cc of rhizosphere soil at the rhizoplane (Foster and Marks, 1967; Malajczuk, 1979) and attract not only other microorganisms (flagellates, amoebae, parasitic bacteria and viruses) but also larger animals such as mites, collembolans, nematodes *etc.* As well as feeding on the rhizosphere microflora, these may also remove the partially decomposed cortical tissues leading to the complete decortication of the root (Head, 1967).

Many of the exudates and lysates escaping from roots are used by the microorganisms in respiration and growth, but some microorganisms secrete new organics which act as secondary sources of energy for other soil inhabitants. With the development of the rhizoflora, microbial gels become inextricably mixed with the root-derived gels so that they are no longer morphologically or biochemically distinguishable: this complex colloidal carbohydrate mix was named mucigel by Jenny and Grossenbacher (1963). Mucigel and microbial polysaccharides have an important role in stabilizing soils (Martin, 1971; Forster, 1979; Gaspari-Mago *et al.*, 1979). Silt sized particles are bound to form larger aggregates by fungal hyphae. Clays become bound onto the surface of small colonies of bacteria both in the rhizosphere (Foster and Rovira, 1978) and bulk soil (Kilbertus and Reisinger, 1978). Some microbial gums are particularly resistant to breakdown (Greenland and Oades, 1975). Even after the death of capsule-producing bacteria, the carbohydrate fibrils may persist in the soil, (Plate 6e) binding the various mineral and organic components of soil crumbs together (Foster, 1981d; Foster *et al.*, 1983). Turchenek and Oades (1978) consider that, within aggregates, bacterial gel is the most important stabilizing agent as it binds clay particles into silt sized aggregates (Plate 6e, g). The binding action of microbial gels varies with the concentration of uronic acids (Martin and Aldrich, 1955).

The rhizosphere microflora Some fix nitrogen; others affect total root length, frequency of laterals, number, length and distribution of root hairs, number of layers in the root cortex and hence root diameter (see Foster, 1984, for review). As well, rhizosphere products release P, K, Fe *etc.* from insoluble minerals (Moghimi *et al.*, 1978).

Except in the surface layers where all the soil may come under the influence of the root, roots may occupy less than 6% of the soil volume. Elsewhere, organic energy sources are

confined to local remnants of organic matter, but despite the oligotrophic conditions in the non-rhizosphere (bulk) soil (Pointdexter, 1981), there are still large populations of microorganisms (10^9E+9/gm), and Clarholm and Rosswall (1980) show that in forest soils their numbers are more than sufficient to account for nearly all the organic C accession each year, and they consider that even under the most favourable conditions, only 15 - 30% of the bacteria were active. Although microbial populations in soil are so large, bacteria occupy less than 0.01% of the total soil surface (Grey *et al.*, 1968). They are not randomly distributed in soils, but are usually associated with substantial organic matter deposits. Thus, Gray *et al.* (1968) showed that in a sandy soil, 60% of the bacteria were attached to organic particles even though these composed only 15% of the soil volume. Similarly in large-area, ultrathin sections of soils Foster *et al.* (1983) showed that the larger (.5 - 1 μm diameter) microbial cells were associated with cellular debris which still contained carbohydrate (Plate 4a, b). Bacteria were also associated with highly lignified materials and occurred sparsely scattered in clay fabrics, but these cells were generally much smaller in diameter (0.3 μm diameter) and contained less stored food reserves such as polyhydroxybutyrate and polyphosphate than similar cells in the rhizosphere (Foster, 1978).

In an extensive ultrastructural study of a single aggregate, Kilbertus (1980) showed that within aggregates bacteria occured within three different types of pore, those with single openings, those with multiple openings and those which were totally enclosed. He also showed that a minimal size of pore could be colonised by bacteria which bore a constant ratio to the diameter of the microorganism. Bacteria within the aggregates greater than 2 mm may experience anaerobic conditions (Greenwood and Goodman, 1967).

Bacterial gels and microbial slimes are recognised by their fibrous (Plate 6b) or granular (Plate 6c) texture, but extensive tracts of amorphous non-0s reactive gels are found in some soil sections (Plate 6a). These may be secreted by earthworms.

Some fungi deposit resistant substances such as melanins in their walls. As well as ribosomes rich in N and P bacteria may deposit storage materials eg. lipids, polysaccharides and polyphosphates in their cytoplasm. The rhizosphere microflora becomes a valuable secondary resource for the soil microfauna. Microfloral use of simple sugars in root exudates causes immobilization of inorganic nitrogen. It has been shown using "microcosms", (small plants growing in a defined medium to which bacteria, amoebae, flagellates, nematodes *etc.* can be added singly or in combination), that addition of predators and browsers, especially mites and nematodes, markedly increases that amount of nitrate nitrogen available to the host plant (Wood *et al.*, 1982; Elliot *et al.*, 1979). Chakraborty *et al.* (1983) showed that soil amoebae attack Gaeumannomyces hyphae so they may be important in the biological control of soil borne plant pathogens.

ORGANICS OF SUBMICRON SIZE

By the time many organic fragments are reduced to micron and sub-micron size they may be so modified morphologically and biochemically that their origin may be obscure. They are then best classified by their ultrastructure and histochemistry. Other particles may retain sufficient characteristic fine structural features that their origin is more certain.

Fibrous or lamellate materials

Many plant cell walls are composed of alternate carbohydrate-rich (electron-transparent) and lignin-rich (electron-dense) lamellae so wall fragments can be recognised on the basis of their distinctive multilamellate structure even when the fragments are less than 1 μm wide, (Plates 5c, g, 6d). Remnants of terminal and middle lamellae are characteristic in their dimensions, texture and electron density and are relatively resistant to decay (Plate 5f, i). As carbohydrate is removed from cell wall fragments and phenolic hydroxyl groups are unmasked, the remnants take up more and more metal ions either from the soil solution (*e.g.*, Mn Fe Al) or from electron dense stains and appear progressively more electron dens. Removal of the carbohydrates brings the elctron dense lignin rich lamellae closer together. Finally only the lignin skeleton remains, distinguishable by its osmiophilic, fibrillar structure.

In contrast, fibrillar remnants which do not react with Os but which are demonstrated by PAMS, PATSP or Au-labeled lectins may be recognised as filamentous polysaccharides such as cellulose microfibrils from higher plant cell wall remnants, or fibrils from the extracellular polysaccharide (ECP) layers of bacteria, actinomycetes or fungi. Some root surfaces are naturally fibrillar (Leppard and Ramomorthy, 1975; Roland, 1971) and Foster (1982) showed that in later stages of decay fibrils from internal cell wall layers are exposed by microbial or physical weathering and make contact with nearby soil minerals.

"Amorphous" or granular materials

Many materials which appear amorphous by light microscopy appear to be granular at the higher resolution of the TEM, especially after suitable histochemical treatment.

Carbohydrates.— Granular deposits in soil fabrics revealed by ruthenium/0s04 or La(OH)3 are probably microbial gels or remnants of root mucilages. In their freshly-formed, fluid state, root mucilages flow freely into pores of submicron size in clay fabrics (Plate 2d) and if these pores are too small to admit bacteria they may be physically protected by the clay from microbial decay. Some of these deposits enclosed between clay tactoids are less than 0.5 μm across (Plate 8g, h) (Foster 1981a, Emerson *et al.*, in press), and although the individual deposits are of small volume, they may be numerous, and thus contain significant energy resources. Other materials reactive to PATSP or PAMS are associated with bacterial walls (Plate 7a, c, g) or are the remains of cell-wall materials of higher plants (Plates 6g, d), but other deposits are not associated with morphologically distinct remnants (Plates 7c, d, 8f). These probably represent fragments of root or microbial ECP. Finch *et al.* (1971) and Griffin (1981) consider that carbohydrate gels can act as water reservoirs. Rovira and Greacen (1957) and Powlson (1980) showed that physically disturbing soils increase their respiration. They proposed that grinding brings bacteria into contact with organic matter from which they were previously physically separated. It is not clear whether mere grinding would be sufficient to expose sub-micron sized deposits. Conformational changes due to dehydration or heating, (as in Australian surface soils), may further reduce the susceptibility of these deposits to decay, and according to Emerson (1977) when polysaccharides are bound to clays they become less succeptible to periodate oxidation. Both sectioned material and isolated clay particles show a very patchy reaction to PAMS (Plate 7d - f) but whether negative reactions are due to absence of carbohydate or to their stabilization on the clay is not clear at present. The complete mineralisation of carbohydrates in and on clays probably depends on their release by grinding in the gizzards of such soil animals as earthworms.

Rybicka (1981) states that periodic acid used in the PATSF reaction specifically oxidises the 1, 2-gycols in polysaccharides to form aldehydes which subsequently react with thiosemicarbazide. The thiol group then reacts with silver to form an electron dense conjugate. The reaction has been widely used in the biomedical sciences to locate carbohydrates, but only rarely on studies of decomposition of organic materials in soils (Foster, 1981a).

The extracellular polysaccharides (ECP) of fungi and bacteria, whether they are granular or fibrillar, can be distinguished by their different resistance to periodate hydrolysis. By treating serial sections of the same soil fabric with periodate for different times before applying the PAMS reagent, Foster (1981a) showed that fungal ECP's were more resistant than those produced by bacteria or roots. Similarly, by incorporating 14C-labeled bacteria and fungi into a grassland soil, Nakas and Klein (1979) showed that bacterial cell walls and polysaccharides were more quickly mineralised than those of the fungi. This may explain why carbohydrates under pastures (rich in root mucilages and bacterial ECP) are more susceptible to periodate degradation than those under forests (Clapp and Emerson, 1972), where most roots are mycorrhizal. Foster (1981c) found that enzymes that removed the cell wall polysaccharides from the thick-walled hyphae of mycelial strands from a forest soil failed to remove the ECP (Plate 8d, e). This may mean that fungal gels are less readily available to soil animals.

Humic substances.— With the fragmentation of cells, polyphenols from cell walls (Harkin 1967) and vacuoles (Zucher, 1983) are released into the soil, so that rhizosphere soils are richer in polyphenols than the bulk soil (Bokhari *et al.*, 1979). Several studies have shown that catalytic polymerisation of phenolic materials by clay minerals occurs to form materials with the properties of humic and fulvic acids (Wang *et al.*, 1980), and humic materials account fo 60 - 70% of soil C (Griffith and Schnitzer, 1975). The importance of these products lies in the N they contain. Ladd (1981) labeled medic plants with both 14C and 15N and showed that 15 - 20% of the 14C was still unmineralised after 4 years but nearly 50% of the 15N remained as stable organic residues. Granular deposits in soils which stain densely with Os, U or Pb are probably "humic" materials rich in polyphenol/protein complexes (Plates 7h, 8g). These may be remnants of vacuolar polyphenolics but they may also represent secondary products of soil microorganisms (Tan *et al.*, 1978), or result from chemical weathering of such materials. Remnants with similar form, internal structure and staining properties as small as tens of nm have been seen in ultrathin sections of soils. These are probably to be identified with humic materials. Early work showed that humic materials formed spheres 60 - 100A in diameter (see van Dijk, 1971, for review). However Chen and Schnitzer (1976), Ghosh and Schnitzer (1982) and Stevenson and Schnitzer (1982) have shown experimentally that the precise ultrastructure of humic compounds *e.g.,* fulvic or humic acid) depends on such environmental factors as pH, water potential and salt concentrations. Thus, the same material may be deposited as sheets, bands or fine fibrils depending on pH (Schnitzer and Kodama, 1976) and this is probably true of similar materials in soils. It might be instructive to fix subsamples of the same soil fabric with aldehydes buffered to different pH's and to observe any difference in form of humic materials. Localized drying, secretion of H^+ ions by roots, or presence of carbonates may cause different deposits of the same material within a few microns of each other in the same soil fabric to have different forms! However it is probable that in nature most humic materials are complexed with clays.

Unfortunately, most of the humic materials in soils cannot be described in precise chemical terms, either because the existing techniques are inadequate for their separation and characterization, or because no two humic molecules are exactly alike (Oades and Ladd, 1977).

Design of specific ultracytochemical techniques is therefore impossible.

Membrane systems

It is not unusual to come across membranes in soils. These are 7 - 10 nm thick and are of variable length. Some are rolled into open or closed tubes and vesicles (Plate 3i (arrows)). These are probably cytoplasmic membranes released from living cells or when bacteria are lysed by bacteriophage. Most cellular membranes contain enzymes; it is possible that whole suites of enzymes, necessary for a sequence of biosynthetic steps which give rise to complex molecules, are preserved *in situ* in such membrane fragments, especially if the membranes roll up or form enclosed vesicles. Such membrane systems may be sites of quite complex biochemical transformations and could give rise to complex nutrients for soil animals.

Enzymes

Martinez and McLaren (1966) remark that, although hundreds of reports dealing with soil enzymes have been published, the question of the origin and localization of these enzymes is still as obscure as it was in the first decade of this century. Burns (1982) has proposed 4 main sites: (1), in the biomass; (2), inside or adsorbed onto cell wall fragments; (3), adsorbed in or on clay minerals; and (4), adsorbed onto or as co-polymers with humic materials. There has been a considerable discussion in the literature as to whether enzyme contents of soils correlate closely with microbial biomass (Nannipieri *et al.*, 1983) or not. Most classes of enzyme have been isolated from soils (Skujins, 1976) but their precise location in soil fabrics is unknown; therefore, the ratio of soil transformations associated with the biomass and with "free" enzymes remains undetermined.

Both roots (Plate 8a) and many soil bacteria excrete enzymes extracellularly where they are associated with their ECP's. (Plates 6b, c, 8b, c). It is also known that enzymes are stabilized by adsorption onto clay surfaces or by forming complex co-polymers with polyphenols. In experimentally synthesized enzyme/polyphenol co-polymers, the enzymes retain much of their catalytic activity and it is likely that similar co-polymers are generated during cellular lysis or after the release of their components into the soil. Enzymes in these complexes may be protected from the action of proteases released by nearby soil microorganisms. Non-enzyme proteins will also be preserved in these sites and may play an important role in animal nutrition.

Although *in situ* ultracytochemical tests for enzymes in soil fabrics have been successfully used to locate enzymes associated with microorganisms (Plate 6b, alkaline phosphatase: Plate 8b, acid phosphatase, Plate 6c catalase, Plate 8c peroxidase) and cellular debris (Plate 6h, acid phosphatase)(Foster, 1981d, 1982), unfortunately, they have so far failed to locate enzymatic activity in or on soil minerals. Some mineral fragments appear occasionally to have unusual electron-dense deposits associated with them after ultracytochemical tests, (Plate 8, b lower arrow). However, because enzyme histochemistry is generally performed on the bulk soil sample, it is impossible to perform experimental procedures and control procedures on adjacent serial sections by present techniques. Moreover since the sections are so thin and the deposits so small (10 - 100 nm) EPMA could not have been used to determine whether the electron dense deposits were fragments of electron dense minerals, or enzyme-specific heavy metal precipitates, so the specificity of these deposits was difficult to establish.

Plate 5. Physically protected organic matter.– (a), and (b). Clay is adsorbed onto the capsule of microorganisms. (c). Fragments of wall material (0) become enclosed in clay aggregates and hence protected from microbial decay. (d), and (e). Humified materials enclosed in a pore within an aggregate. The material appears granular at high magnification (e). (f), and (g). Amorphous (f)and lamellate (g) organic matter (0) enclosed in clay fabrics. (h). Amorphous organic materials mixed with clay. (i). Middle lamella fragment enclosed in clay.

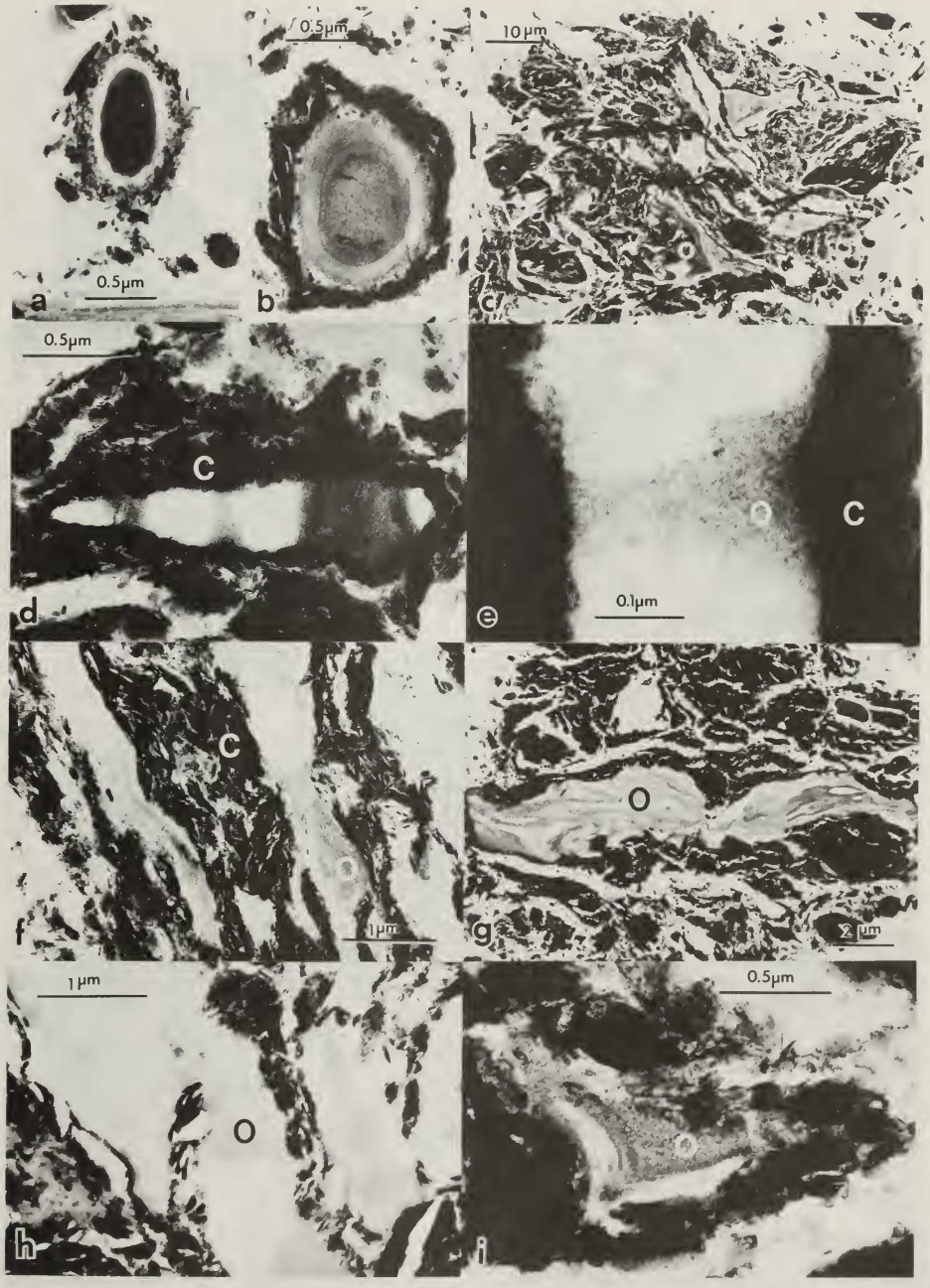

Plate 6. Acidic carbohydrates (lanthanum reactive) in soils.– (a). General view of a fabric containing amorphous (Ca) acidic carbohydrates and cell wall fragments. (b). Test for alkaline phosphatese locates the enzyme in microbial capsules. (c). Catalase is also associated with capsule materials. (d). Detail of (a) showing a small colony of bacteria (B) supported by amorphous and laellate organic matter (0). (Lanthanum stain). (e). Even after a bacterial colony (B) has died the capsule materials persist linking other soil components such as humic materials (arrow) together. (f), and (g). Fibrous (f) and amorphous (g) lanthanum reactive organic materials in clay fabrics (arrows). (h). After the acid phosphatase reaction some organic particles (0) appear to have to have enhanced electron density. (i). Membrane (arrows), possibly remains of plant cell walls abound in many soils.

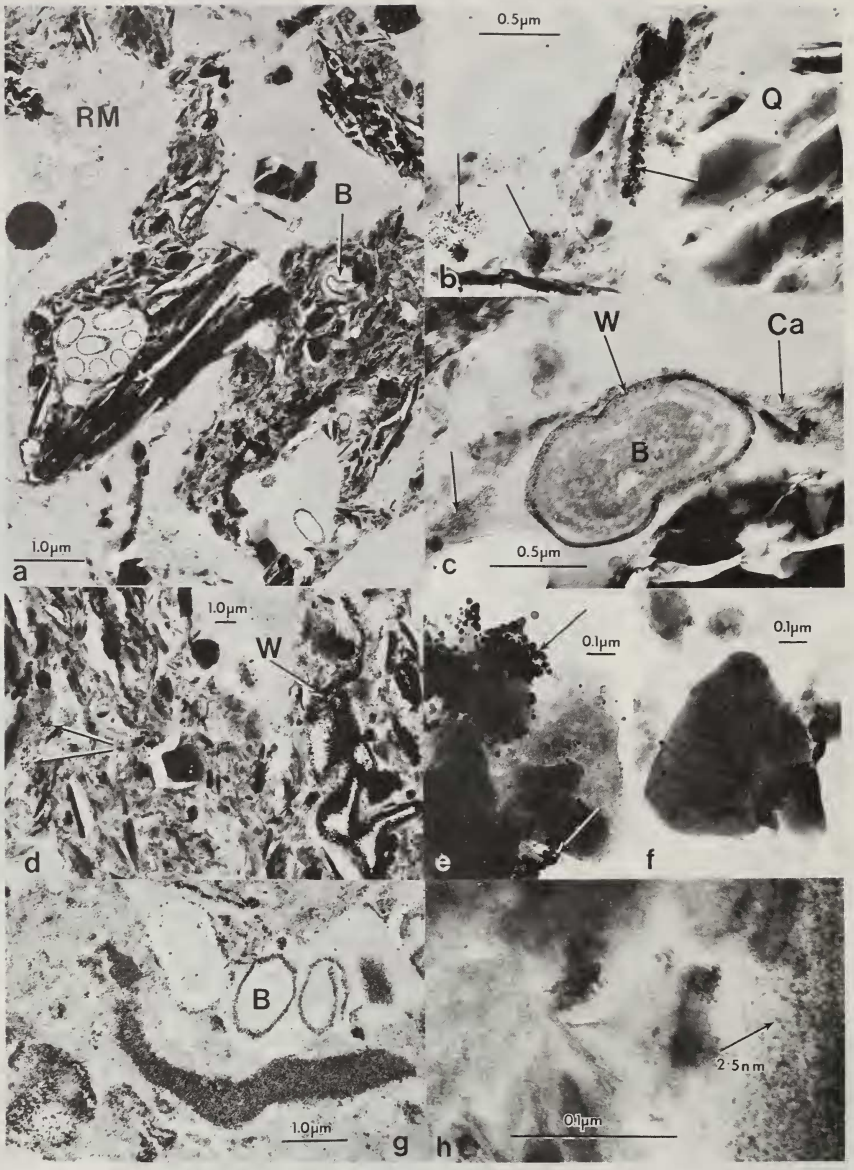

Plate 7. Neutral carbohydrates (PAMS AND PATSP-reactive) in soils.– (a). PAMS stains the cell walls of bacteria (B) but not their capsule carbohydrates or the root mucilage (RM). (b). At higher magnification the granular product of the PAMS reagents is associated with linear, presumably wall materials, round objects, perhaps bacteria and clay particles (arrows). (c). Similarly the PATSP reagents stain the cell wall (W) of bacteria (B) as well as amorphous materials (Ca, arrow) in the soil. (d). Wall (W) and other organic deposits stained by PAMS. (e), and (f). If clay particles are isolated from soils and tested with the PAMS reagents only certain particles are stained (arrows), as when sections are stained. This suggests that only a few clay particles are coated with carbohydrate. (f) is the control which has been treated with silver methenamine but not with the periodate. (g). PAMS treated "humus" from a compost heap. The material varies widely in its reaction to the stain; presumably the particles which are less intensely stained are devoid of materials that are readily periodate-reactive. (B) indicates bacteria. (h). At high magnification 0s-treated materials contain granules 25 nm in diameter. These are probably humic materials.

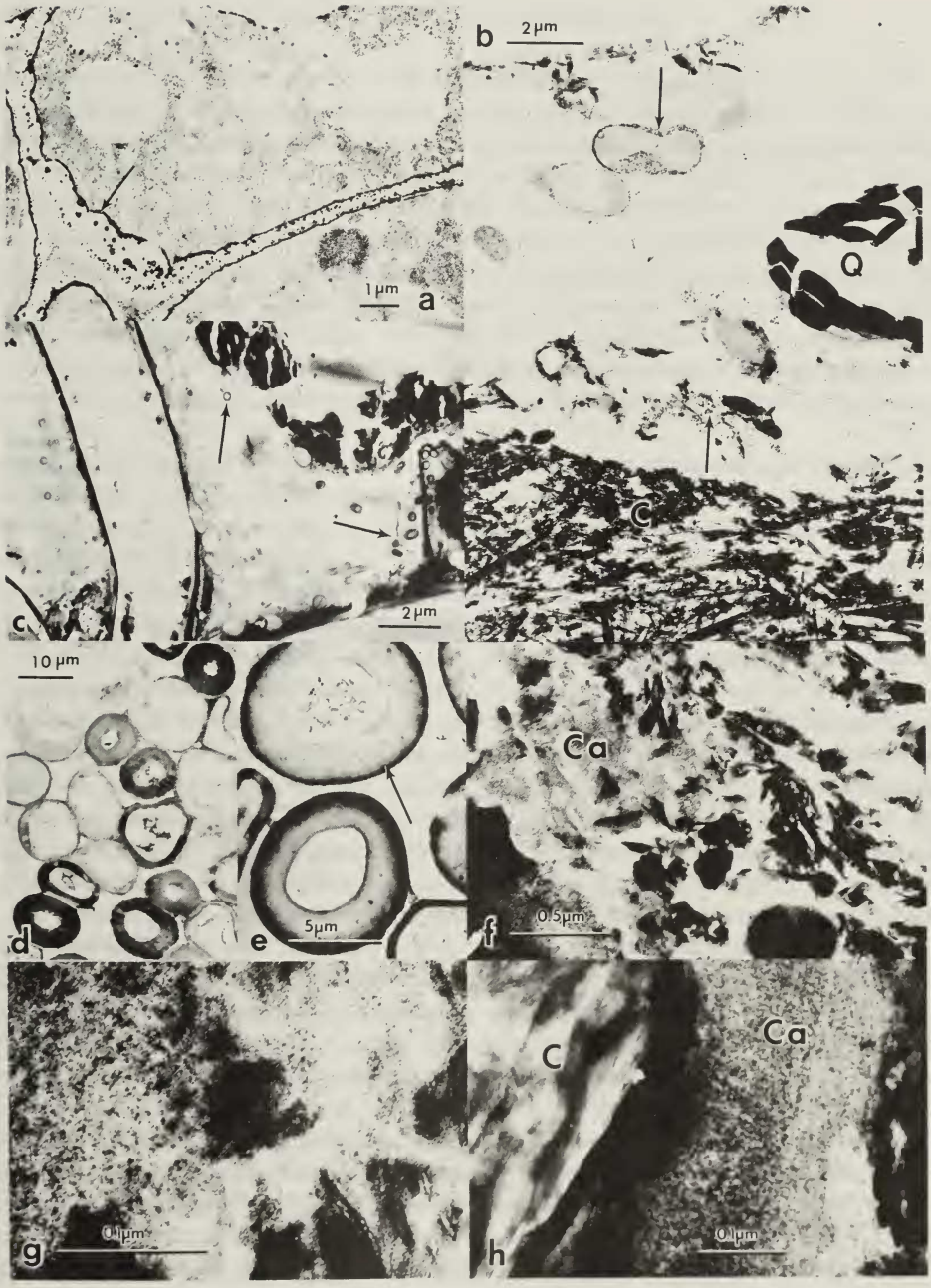

Plate 8. Various histochemical reactions.– (a), and (b). In plant cells (a) acid phosphatase is associated with the plasmalemma and cell wall. In soils (b) acid phosphatase is generally associated with bacteria, though usually only a few react. Electron density is sometimes also associated with amorphous materials (lower arrow). (c). In waterlogged soils peroxidase is associated with narrow tubular microorganisms (arrows) though, again, only a few of the cells are reactive. (d), and (e). The thick walled hyphae of mycelial strands in soil stain intensely throughout (d) but if the cells are pretreated with amylase, only the outer layers of the cell walls still stain. (f), (g), and (h). The final product of organic matter transformations in soils are amorphous materials. These are usually mixed in composition since they stain with PATSP (neutral carbohydrates) (f), osmium alone (humic materials) (g) and ruthenium red/osmium (acidic carbohydrates) (h).

SUMMARY AND CONCLUSIONS

There are three reasons why ultracytochemical studies of soil organics are difficult. First, the hardness of minerals imposes severe technical restraints; second, electron micrographs are usually monochrome; and third, organic materials in soils are chemically complex and much modified from their original structural and biochemical properties.

Dommergues *et al.* (1977) consider that TEM is useless for quantifying materials in soils because of the sophisticated and lengthy procedures involved in sample preparation, and difficulties with respect to microscopic field orientation and size. The great resolution of the TEM has its premium in the extremely small area examined, so that where quantification may be attempted, sample variability is the dominant consideration. The role of TEM ultracytochemistry is, rather, investigation of the structure environment of organic matter at stages of mineralization which are determined by prior quantitative biochemical or biophysical studies.

Although image processing can be used to produce colored displays of electron images, *e.g.*, distinguish between electron density due to background osmium reaction and that due to an histochemical reaction on the basis of element distribution (Tanaka and Mitsushima 1984), most electron micrographs contain only black, white and grey areas. In general only those products which are electron-dense can be detected, *i.e.*, minerals and materials containing heavy metals. It is, therefore, much more difficult to design ultracytochemical reactions for electron microscopy than cytochemical reactions for light microscopy, where colored stains markedly enhance the visibility of the products of histochemical reactions. Ultrathin sections of most biological tissues have little or no intrinsic electron density, therefore, the results of histochemical tests are unequivocal, providing adequate controls are employed in which target groups are masked or destroyed. These ideal conditions do not hold for ultrathin sections of soils. Here the minerals are electron-dense to varying degrees; organic matter may adsorb electron-opaque materials from nearby minerals. Moreover, although histochemcial reagents give uniquivocal results when tested against relatively pure and well characterised materials in tissues, the same may not be true of the much modified materials which occur in soils.

Until recently, histochemical reagents were not very specific. The use of antibodies and lectins labeled with heavy metals, (Knox and Clark, 1978) may prove very useful in the study of mineralization of organic matter though preliminary experiments showed little sign of specific staining of rhizospheres (Foster, unpublished). Lectins may almost prove to be too specific in that they may detect only relatively unmodified materials which can be recognised anyway from their structure or location.

Ultrahistochemical analysis of soil fabrics is important because it provides information not easily obtained by other electron optical techniques. Thus ultracytochemistry not only tells us where organics are located in soil fabrics, but also something of their biochemical properties. EPMA, SIMS, LAMMA *etc.* are useful in that they tell us what elements, ions or chemical groups are present in organic deposits; they do not tell us how these parts are put together to form an organic complex.

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